

(51) International Patent Classification <sup>4</sup> :  C12N 1/26, 5/01	A1	(11) International Publication Number:  WO 86/ 03773  (43) International Publication Date:  3 July 1986 (03.07.86)
--	----	--

**Published**  
*With international search report.*

**BEST AVAILABLE COPY**

***FOR THE PURPOSES OF INFORMATION ONLY***

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GA	Gabon	MR	Mauritania
AU	Australia	GB	United Kingdom	MW	Malawi
BB	Barbados	HU	Hungary	NL	Netherlands
BE	Belgium	IT	Italy	NO	Norway
BG	Bulgaria	JP	Japan	RO	Romania
BR	Brazil	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	LI	Liechtenstein	SN	Senegal
CH	Switzerland	LK	Sri Lanka	SU	Soviet Union
CM	Cameroon	LU	Luxembourg	TD	Chad
DE	Germany, Federal Republic of	MC	Monaco	TG	Togo
DK	Denmark	MG	Madagascar	US	United States of America
FI	Finland	ML	Mali		
FR	France				

PROCESS FOR INCREASING THE SOLUBILITY OF GASES IN AN  
AQUEOUS MEDIUM AND AN EMULSION FOR CARRYING OUT  
SAID PROCESS

The present invention relates to a process for increasing the solubility of gases in an aqueous medium and an emulsion for increasing the solubility of gases in an aqueous medium.

5       The low solubility of oxygen in water presents a problem in biotechnical processes which depend on oxygen concentration. This is especially the case when enzymes or cells are used for enzymatic conversions requiring oxygen as co-substrate. Also cell  
10 growth within immobilised systems is often slowed down due to diffusional in and out hindrance of gases, notably oxygen.

Attempts at solving this problem have been made. Thus, a photosynthetically active alga has been co-  
15 immobilised with a microorganism catalysing the oxidative deamination of amino acids to  $\alpha$ -keto acids with L-amino acid oxidase (P. Wikström, E. Szwajcer, P. Brodelius, K. Nilsson, K. Mosbach (1982) "Formation of h-keto acids from amino acids using immobilized bacteria and algæ", Biotechnol. Lett. 4:153-158).  
20 Alternatively, a high catalase activity in a microorganism has been used to convert hydrogen peroxide in situ into oxygen (O. Holst, S.O. Enfors, B. Mattiasson (1982) "Oxygenation of immobilized cells using hydrogen-peroxide; a model study of Gluconobacter oxydans converting glycerol to dihydroxyacetone", Eur. J. Appl. Microbiol. Biotechnol. 14:64-68). Furthermore, manganese dioxide (P. Brodelius, K. Nilsson, K. Mosbach  
25 (1981) "Immobilized cells of Trigonopsis variabilis containing D-amino acid oxidase", Appl. Biochem. Biotechnol. 6:293-308) as well as charcoal (E. Szwajcer, P. Brodelius, K. Mosbach (1982) "Immobilized whole  
30

cells of *Providencia* sp. PCM 1298 containing L-amino oxidase", *Enzyme Microb. Technol.* 4:409-413) have been co-immobilised with microorganisms both to eliminate hydrogen peroxide and, at the same time, to produce  
5 oxygen.

Literature also reports on the addition of oxygen carriers, such as perfluoro compounds, to the medium (P. Adlercreutz, B. Mattiasson (1982) "Oxygen supply by hemoglobin or emulsions of perfluorochemicals",  
10 *Eur. J. Appl. Microbiol. Biotechnol.* 16:165-170).

However, none of these techniques has proved to be sufficiently efficient to improve to any essential extent the gas transport into and out of cells.

The present invention provides a novel process and  
15 a means for increasing the solubility of gases in an aqueous medium, thereby to facilitate the supply of, for example,  $O_2$  to cells and the removal of  $CO_2$  from cells.

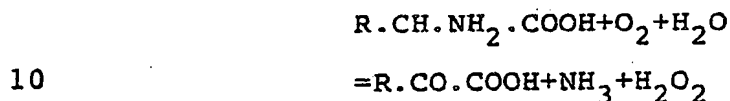
The process according to the invention is characterised in that there is added to the aqueous medium  
20 a silicone emulsion in the form of an aqueous emulsion of a copolymer of a silicone and a hydrophilic compound, optionally in mixture with a silicone. This silicone emulsion functions as a gas carrier and comprises  
25 silicone compounds which are nonpolar, chemically inert and have high solubility for gases like oxygen and carbon dioxide. Since these silicone compounds are insoluble in water, they must, for the purpose of the present invention, be copolymerised with strongly hydrophilic compounds. These copolymers readily  
30 form small micelles, in the nonpolar interior of which substances such as oxygen may be retained.

To further increase the effect of the copolymer emulsion, silicone may be added thereto.

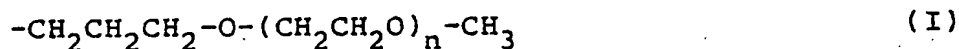
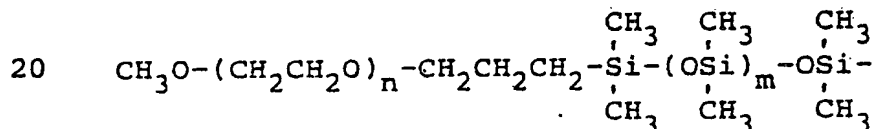
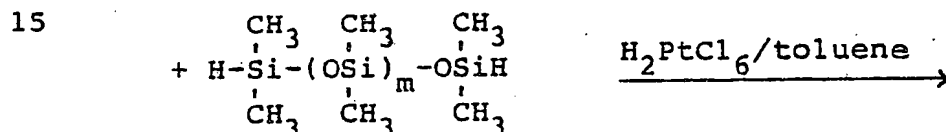
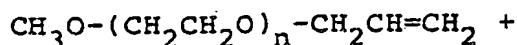
The silicone emulsion according to the invention  
35 is characterized in that it comprises a copolymer of a silicone and a hydrophilic compound, optionally in mixture with a silicone.

In the following Examples, use is made, as a model system, of immobilised cells of *Providencia* sp. PCM 1298 (E. Szwajcer, P. Brodelius, K. Mosbach (1982) "Immobilized whole cells of *Providencia* sp.

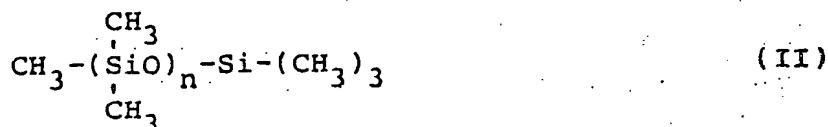
- 5 PCM 1298 containing L-amino acid oxidase", *Enzyme Microb. Technol.* 4:409-413). These cells catalyse the following transformation:



The copolymer employed was a copolymer between polydimethyl siloxane and polyethylene oxide:



- 25 The silicone employed was:



30

#### MATERIALS AND METHODS

- Chemicals: Alginate was obtained from Alginate Industry (Girvan, U.K.). L-Methionine was obtained from Merck (Darmstadt, Federal Republic of Germany). Polydimethyl
- 35 siloxanes as well as perfluorodecalin were purchased from Fluka (Buchs, Switzerland) and "Pluronic F68" from Serva (Heidelberg, Federal Republic of Germany).

All other chemicals were of analytical grade and obtained from diverse commercial sources. The copolymer was prepared by hydrosilylation with silicones containing a Si-H end group and polyethylene oxide (PEO)

5 functionalised with an allyl end group (see formula I).

Details of the preparation of the copolymer have previously been described by Kendrick et al (T.C. Kendrick, B.M. Kingston, N.C. Lloyd, M.J. Owen (1967) "The surface chemistry of polyurethane foam formation. 1. Equili-

10 brium surface tensions of polysiloxane-polyether block copolymer solutions", J. Coll. Interface Sci. 24:135-140).

Preparation of the emulsions: The aqueous phase (buffer or growth medium), silicone and copolymer were mixed and autoclaved for 20 min. The amount of copolymer

15 employed was always 5% by weight of the total volume.

The silicone contents were 5, 10, 15 and 25% by volume, respectively, of the total volume. The mixture was then sonicated (5 min, 350 V) using as probe a "A 350 G" sonicator from Ultrasonic Ltd., U.K.

20 The measurement of oxygen solubility was done according to Leonhardt (A. Leonhardt (1984) Thesis, University of Freiburg). The viscosity was determined with a Brookfield Synchro-Lectric Viscosimeter (Brookfield E. Laboratories, Straughlan, USA).

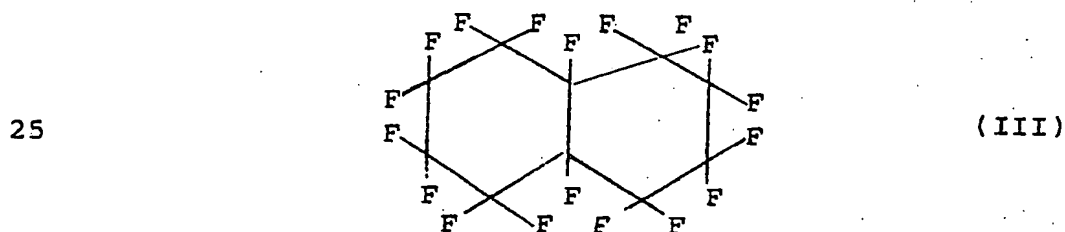
25 Cultivation of Providencia sp. PCM 1298: Fermentation of the bacteria was performed essentially as previously described (E. Szwajcer, P. Brodelius, K. Mosbach (1982) "Immobilized whole cells of Providencia sp. PCM 1298 containing L-amino acid oxidase", Enzyme Microb. Technol. 4:409-413).

30 Enzym assay: The amino acid oxidase activity within whole cells of Providencia was assayed either by a colorimetric method using dinitrophenyl hydrazine or reversed phase HPLC. In all cases, L-methionine was used as substrate. The corresponding  $\alpha$ -keto- $\gamma$ -methiol butyric acid formed by the amino acid oxidase present in the cells was established by these two

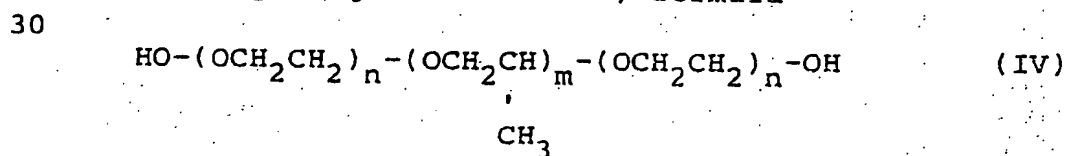
methods ((P. Brodelius, K. Nilsson, K. Mosbach (1981). "Immobilized cells of *Trigonopsis variabilis* containing D-amino acid oxidase", Appl. Biochem. Biotechnol. 6:293-308).

- 5 Enzyme stability test: This test was performed with suspended free cells of *Providencia*. One sample was kept in 50 mM Tris-HCl buffer, pH 7.5, alone, while the other was substituted with 10% by volume silicone and 5% by weight copolymer in the same buffer solution. The two samples in the test tubes with screw caps were incubated on a rocking table for 2 days at 30°C.

10 Cultivation of immobilised cells: Beads (diameter 2 mm) containing cells of *Providencia* (2% by weight cells) were packed in a small reactor (total volume 5 ml), and growth medium containing 10% by volume silicone and 5% by weight copolymer were saturated continuously with pure oxygen (20 ml/min.) and pumped continuously through the reactor (8 ml/h). In a parallel experiment, growth medium containing 10% by volume perfluoro-decalin, formula



and 5% by weight "Pluronic", formula



35 was pumped through the reactor. Furthermore, growth medium alone was pumped through the reactor as a reference. Cultivation was carried out at 28°C for 15 h.

Test of cell viability and L-amino acid oxidase activity: About 50 beads cultivated in the reactor were taken out and solubilised in 0.1 M sodium phosphate buffer (5 ml, pH 7.5). After shaking for 2 h at room temperature, the beads were completely solubilised. After centrifugation (10 min., 10,000 g), the cells were examined. The viability of the cells was determined with an oxygen electrode. As a measure of cell growth, the protein content of the sample was used, obtained by the method of Lowry et al (O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randall (1951) "Protein measurements with the Folin-phenol reagent", J. Biol. Chem. 193:265-275). The amino acid oxidase activity was assayed according to the above procedure.

The invention will be illustrated in more detail in the following Examples in conjunction with the drawings.

Fig. 1 shows the result of a L-amino acid oxidase stability test on free cells of *Providencia* sp. PCM 1298. Fig. 2 shows the effect on the L-amino acid oxidase activity due to different concentrations of oxygen-carrying additives and cells in alginate beads. Fig. 3 shows the effect of different silicone additives to 5% by weight copolymer on the relative enzyme activity due to different cell concentrations in the alginate beads. Fig. 4 shows the effect of two different oxygen carriers on the L-amino acid oxidase activity due to the cell concentration in the alginate beads. Fig. 6 shows the effect of different concentrations of oxygen carrier on the relative viscosity  $\eta_{rel}$  at 20°C. Fig. 6 shows the oxygen solubility at 37°C in different substances.

#### EXAMPLE 1

##### Enzyme stability

This test was performed to show any negative effect due to the presence of copolymer or silicone on the amino acid oxidase activity in the bacteria.



Cell samples were taken at different intervals, washed with 50 mM Tris-HCl, pH 7.5, and then incubated with 5 mM L-methionine in the same standard buffer. The amount of  $\alpha$ -keto acid formed was measured by a colorimetric method using 2,4-dinitrophenyl hydrazine.

As will appear from Fig. 1, both samples showed the same relative activity after 3 h incubation of the cells. After 22 h, the activity in the reference sample had decreased to about 87%, whereas the activity in the sample containing silicone and copolymer remained practically unchanged. After 40 h, a decrease of the activity was noted in both samples, but the sample with silicone and copolymer showed a higher remaining activity. The result of this test shows that the L-amino acid oxidase activity did not decrease in the presence of the polymers.

#### EXAMPLE 2

Cell growth in alginate beads and enzyme activity after incubation in growth medium in the presence of different oxygen-carrying additives

The effect of a mixture of 10% by volume silicone and 5% by weight copolymer on the cell growth and the amino acid oxidase activity was determined. The initial concentration of the cells within the beads was 2% by weight. The total reactor volume was 5 ml. The growth medium was pumped through the reactor (8 ml/h) and saturated continuously with pure oxygen gas (20 ml/min.).

As will appear from Table 1, the cell growth in alginate beads increased somewhat after 15 h. In analogy therewith, also the amino acid oxidase activity increased to some extent. With the medium containing perfluorodecalin and "Pluronic" these values showed a slight increase. On the other hand, the medium enriched with silicone and copolymer in accordance with the present invention showed a drastic increase in growth and enzyme activity.

TABLE 1

Preparation	Incubation time h	Protein content in cells from 50 alginate beads (mg.ml <sup>-1</sup> )	Relative O <sub>2</sub> consumption (%)	Relative enzymatic activity (%)
Samples at 0 h	0	20	100	100
Reference sample <sup>a</sup>	15	24	110	110
PFD sample <sup>b</sup>	15	30	150	140
Silicone sample <sup>c</sup>	15	100	450	420

<sup>a</sup>Reference, growth medium free from oxygen carrier

<sup>b</sup>PFD, growth medium containing 10% by volume perfluorodecalin and 5% by weight "Pluronic".

<sup>c</sup>Silicone, growth medium containing 10% by volume silicone and 5% by weight copolymer.

### EXAMPLE 3

The effect of different concentrations of oxygen-carrying additives and cells present in alginate beads, on the L-amino acid oxidase activity

Four different cell concentrations in alginate beads were used, ranging from 2 to 8% (Fig. 2). The beads (diameter 2 mm) were present in a small column (total volume 2 ml). 5 mmol L-methionine in 50 mM Tris-HCl buffer, pH 7.5, were used as substrate. In all tests, the solutions or emulsions were saturated with pure oxygen. Different oxygen carriers (see Fig. 2) were added to the substrate solution. Neither silicone nor perfluorodecalin alone could be used since they are not soluble, nor is it possible to obtain any emulsions thereof. Pure oxygen gas was used to saturate all solutions. The enzymatic activity of the beads

containing 2% by weight cells is defined as the relative activity "1" for all activity tests using 5 mM L-methionine in 50 mM Tris-HCl buffer, pH 7.5.

5 Samples were taken after 90 min. of incubation and analysed with HPLC (P. Brodelius, K. Nilsson. K. Mosbach (1981) "Immobilized cells of *Trigonopsis variabilis* containing D-amino acid oxidase", Appl. Biochem. Biotechnol. 6:293-308).

10 In the case of the reference sample, an increase of the said concentrations led to an increase of measured L-amino-acid oxidase activity by a factor of 1.8. Using "Pluronic" which is a common emulsifier for perfluoro chemicals, no influence on the enzyme activity was observed. The presence of copolymer alone  
15 increased the oxygen-carrying capacity. Most likely, the silicone part of the copolymer molecule is responsible for the observed increase (see also Fig. 6). The structure of "Pluronic" resembles that of polyethylene oxide which is a part of the copolymer (I).  
20 Thus, it is the silicone part which is responsible for the oxygen-carrying capacity here expressed as increased oxidase activity. The addition of silicone to the emulsion of the copolymer in water increases the amino acid oxidase activity (see Fig. 3), presumably because it increases the oxygen solubility  
25 (see also Fig. 6). The effect of silicone together with copolymer is more pronounced when the cell density increases in the beads (Fig. 4).

30 A clear difference in L-amino acid oxidase activity for higher concentrations of cells is shown between the sample containing copolymer together with silicone than for perfluorodecalin together with "Pluronic" (see Fig. 4). Since relatively high concentrations of these acid carriers are required to obtain  
35 significant effects, there is a risk of a viscosity increase. As will appear from Fig. 5, the silicone increases the viscosity only slightly, even at a con-

centration of 25% by volume, in contrast to the effect which perfluorodecalin has on the viscosity.

### Conclusions

5 The use of silicone copolymers as herein described is of great potential interest to a number of biotechnical processes, particularly those which depend on oxygen concentration. Thus, silicone emulsions according to the invention may be used to facilitate the gas transport process in bacterial cells, yeast fungus  
10 cells, plant cells and animal cells.

The advantage which silicone gives over perfluoro chemicals is, inter alia, its low effect on the viscosity, allowing high concentrations as well as a low price. As indicated in the Examples, the cell growth  
15 in the present model system is far more efficient than with perfluoro compounds.

Added separately, silicone and "Pluronic" also lead to a substantial increase in oxygen solubility. However, only the copolymers described herein lead  
20 to stable micellar solutions maintaining the oxygen entrapped. Since the production of these compounds from inexpensive raw materials is relatively simple, the compounds are highly useful for both immobilised and free microorganisms, plant and animal cells.

## CLAIMS

1. A process for increasing the solubility of gases in an aqueous growth medium, c h a r a c t e r i s e d in that there is added to the medium a silicone emulsion in the form of an aqueous emulsion of a copolymer of a  
5 silicone and a hydrophilic compound, optionally in mixture with a silicone.

2. A process as claimed in claim 1, c h a r a c -  
t e r i s e d in that the hydrophilic compound in the copolymer is polyethylene oxide.

10 3. A process as claimed in claim 1, c h a r a c -  
t e r i s e d in that the silicone is polydimethyl siloxane.

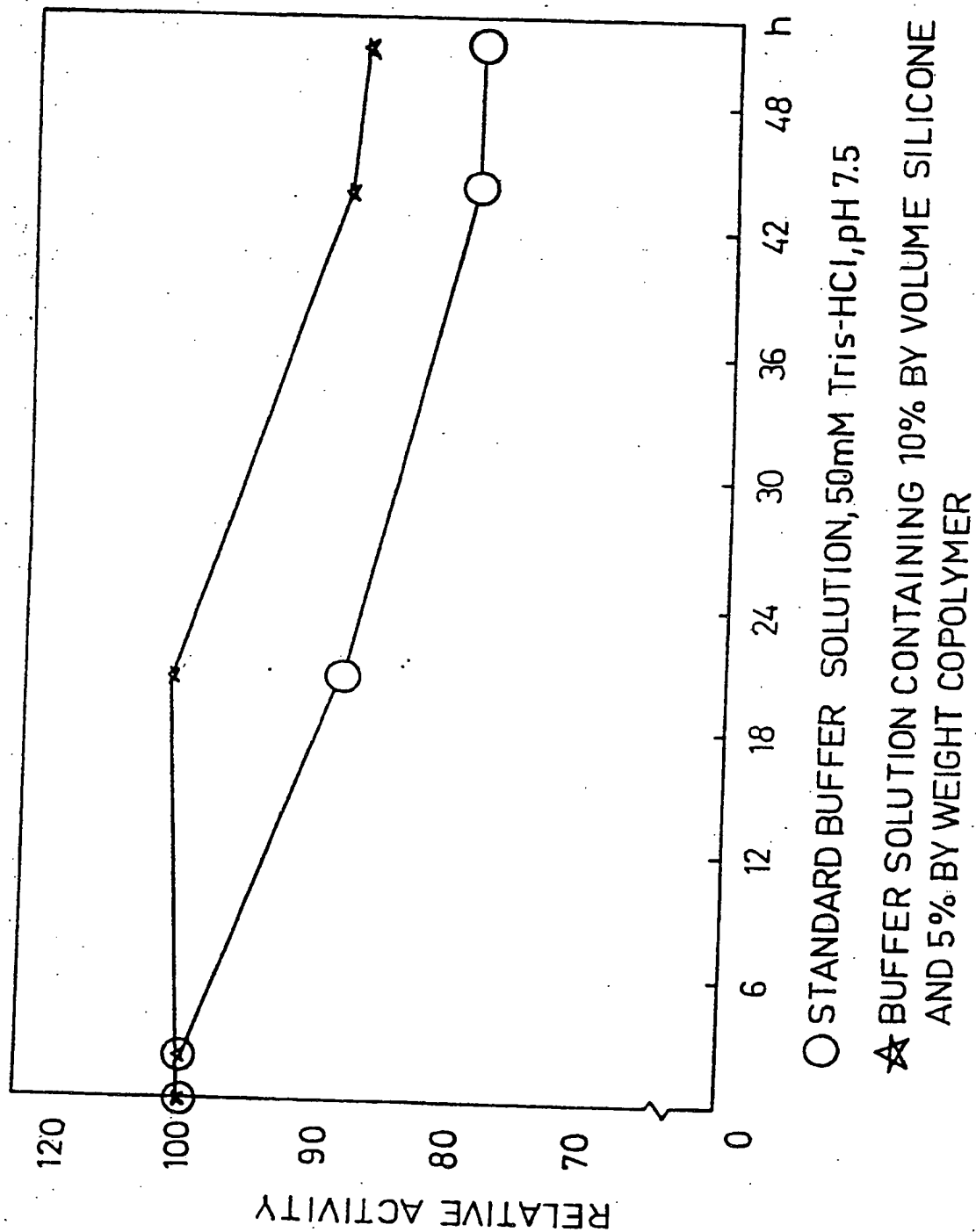
4. A silicone emulsion for increasing the solubility of gases in an aqueous medium, c h a r a c -  
15 t e r i s e d in that it comprises a copolymer of a silicone and a hydrophilic compound, optionally in mixture with a silicone.

5. A silicone emulsion as claimed in claim 4,  
c h a r a c t e r i s e d in that the hydrophilic  
20 compound is polyethylene oxide.

6. A silicone emulsion as claimed in claim 4 or 5, c h a r a c t e r i s e d in that the silicone is polydimethyl siloxane.

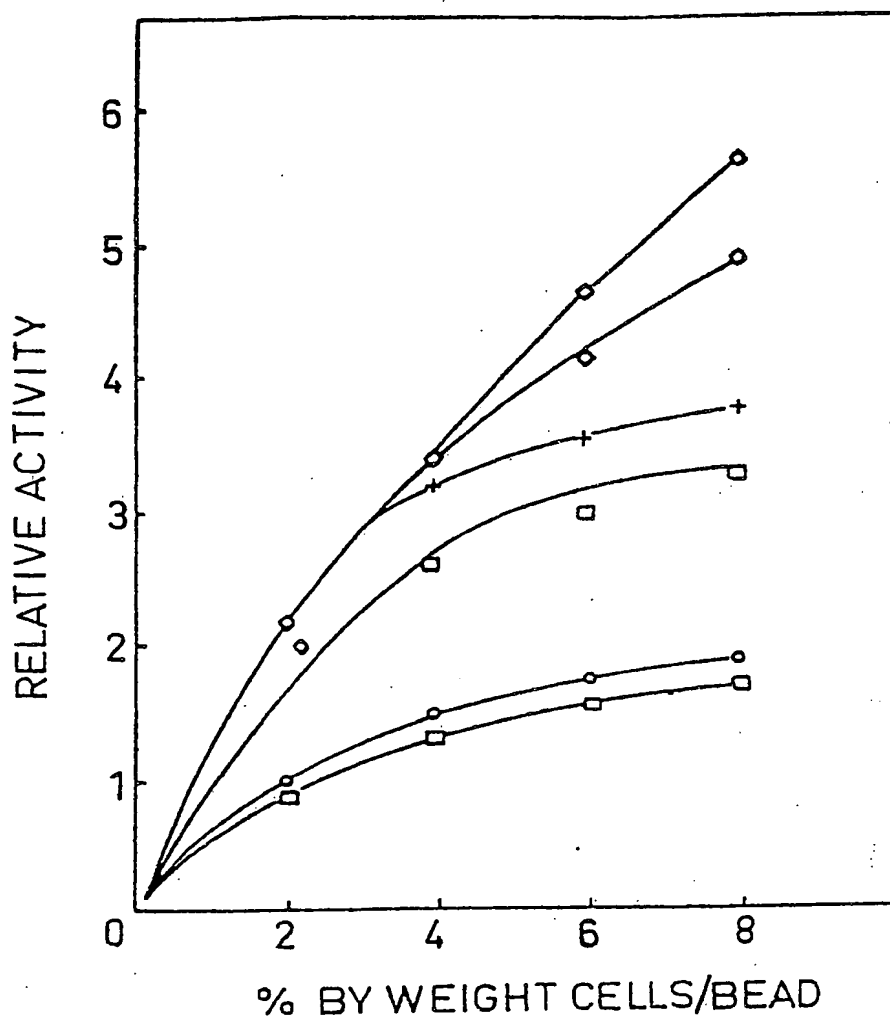
1/6

Fig.1



2/6

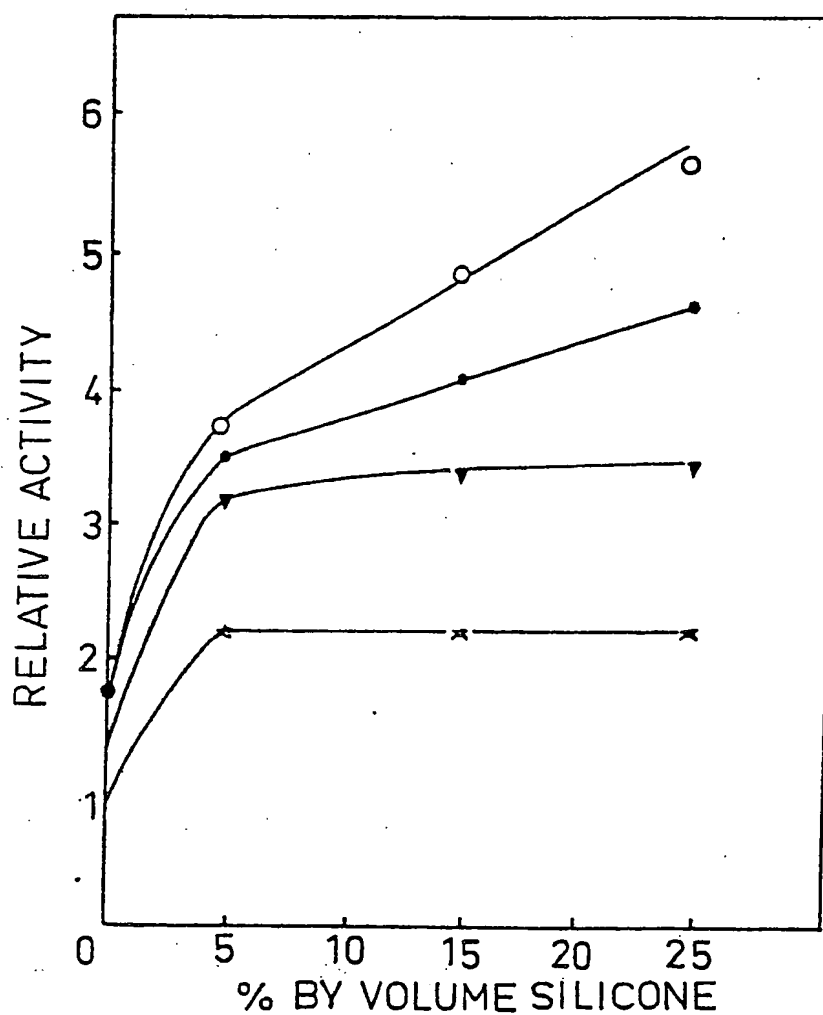
Fig.2



- SUBSTRATE ALONE IN BUFFER SOLUTION
- SUBSTRATE SOLUTION CONTAINING 5 % BY WEIGHT "PLURONIC"
- SUBSTRATE SOLUTION CONTAINING 5 % BY WEIGHT COPOLYMER
- + SUBSTRATE SOLUTION CONTAINING 5% BY VOLUME SILICONE AND 5% BY WEIGHT COPOLYMER
- ◇ SUBSTRATE SOLUTION WITH 15% BY VOLUME SILICONE AND 5% BY WEIGHT COPOLYMER
- ◆ SUBSTRATE SOLUTION WITH 25% BY VOLUME SILICONE AND 5% BY WEIGHT COPOLYMER

3/6

Fig.3

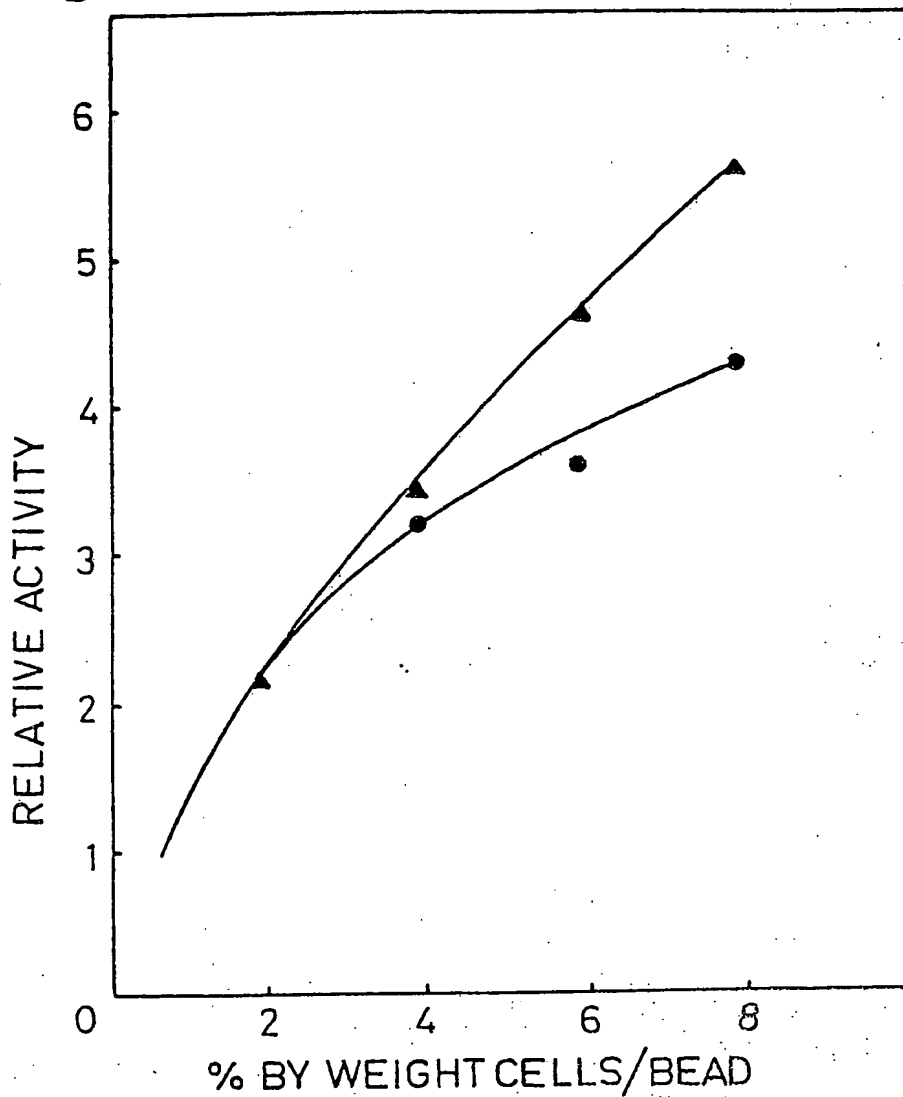


- ★ 2 % BY WEIGHT CELLS IN ALGINATE BEADS
- ▼ 4 % BY WEIGHT CELLS IN ALGINATE BEADS
- 6 % BY WEIGHT CELLS IN ALGINATE BEADS
- 8 % BY WEIGHT CELLS IN ALGINATE BEADS



4/6

Fig.4

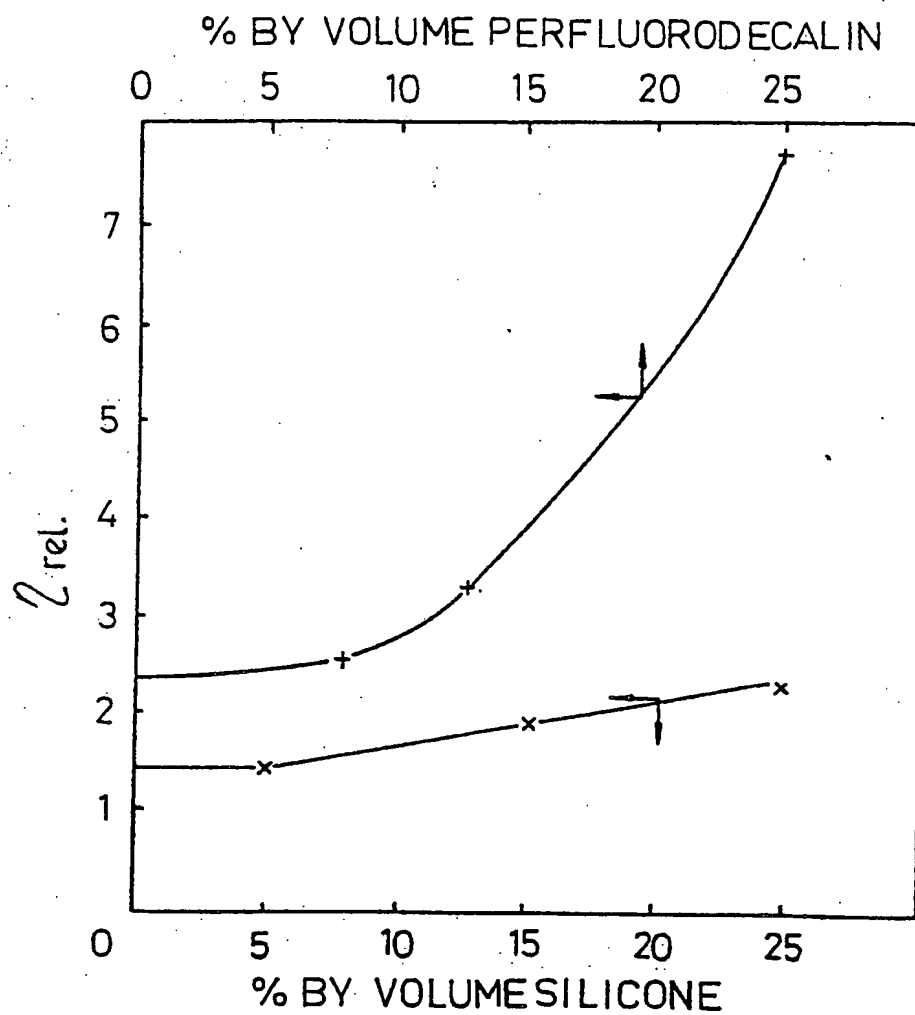


▲ SUBSTRATE SOLUTION CONTAINING 5% BY WEIGHT COPOLYMER AND 25% BY VOLUME SILICONE

● SUBSTRATE SOLUTION CONTAINING 5% BY WEIGHT "PLURONIC" WITH 25% BY VOLUME PERFLUORODECALIN

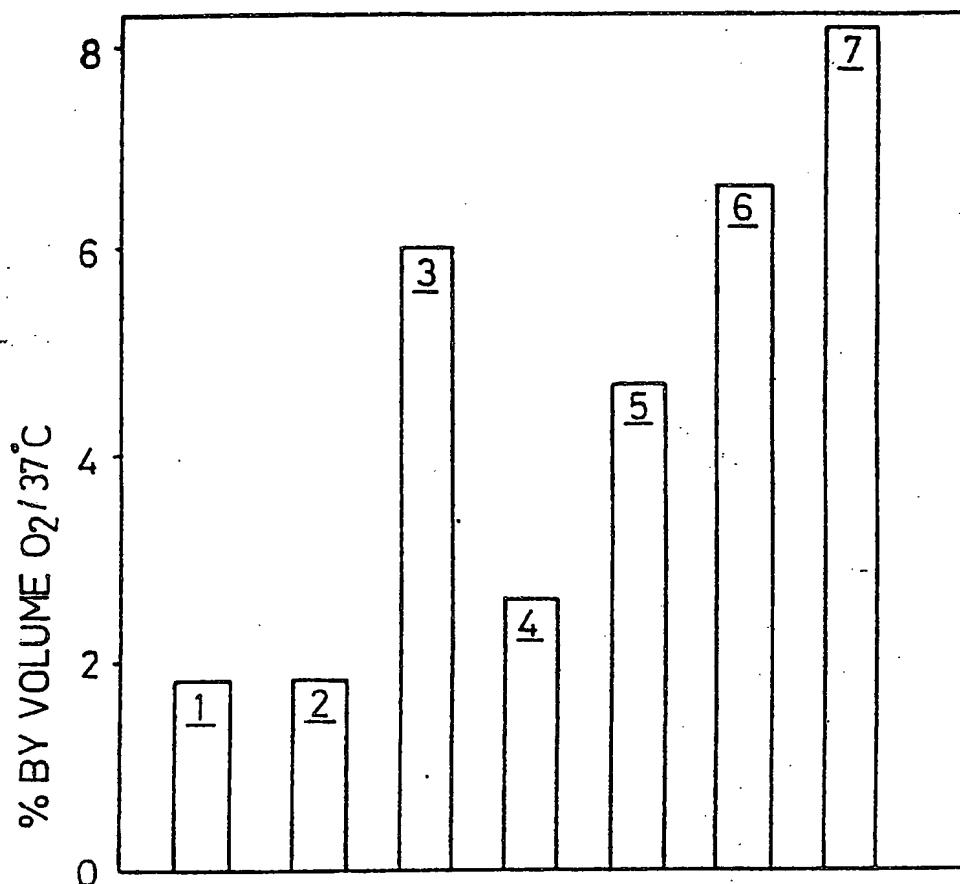
5/6

Fig.5



6/6

Fig.6



1=H<sub>2</sub>O 2=5% "PLURONIC"

3= 5% "PLURONIC"+25% PERFLUORODECALIN

4= 5% COPOLYMER + H<sub>2</sub>O

5= 5% COPOLYMER+5% SILICONE

6= 5% COPOLYMER+15% SILICONE

7= 5% COPOLYMER+25% SILICONE

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/SE85/00536

<b>I. CLASSIFICATION OF SUBJECT MATTER</b> (If several classification symbols apply, indicate all) *		
According to International Patent Classification (IPC) or to both National Classification and IPC 4		
C 12 N 1/26, C 12 N 5/01		
<b>II. FIELDS SEARCHED</b>		
Minimum Documentation Searched 7		
Classification System	Classification Symbols	
IPC 3	C 12 N 5/01	
IPC 4	C 12 N 1/00, 5/00, /02, 1/12, /14, /16, /20, /26, /28, /38; C 08 L 83/12 .../...	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched *		
SE, NO, DK, FI classes as above		
<b>III. DOCUMENTS CONSIDERED TO BE RELEVANT *</b>		
Category *	Citation of Document, 11 with indication, where appropriate, of the relevant passages 12	Relevant to Claim No. 13
Y	FR, A, 2 177 051 (TANABE SEIYAKU CO LTD) 2 November 1973 See page 2 line 11 and page 3 line 9-11 & DE, 2314631 US, 3850753 GB, 1409944 JP, 48096782	1-3
Y	SE, B, 403 433 (THE GREEN GASS CORP, OSAKA JP) 6 April 1975 & NL, 7402664 FR, 2246262 DE, 2404564 AU, 65200/74 US, 3962439 GB, 1445925 AT, 333962 US, 3993581 AU, 471724 CH, 598749 .../...	1-3
<p>* Special categories of cited documents: 10</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&amp;" document member of the same patent family</p>		
<b>IV. CERTIFICATION</b>		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
1986-03-04	1986-03-07	
International Searching Authority	Signature of Authorized Officer	
Swedish Patent Office	Yvonne Siösteen	

## FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

II Fields searched (cont).

US C1 195:1, 82, 100, 112;  
435:240-241, 243-249, 253-255, 257,  
 258

V. ☐ **OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE**

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers ..... because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim numbers ..... because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim numbers ..... because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ **OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING**

This international Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the international Searching Authority did not invite payment of any additional fee.

## Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
	CA, 1042345 JP, 50069219 SE, 7400989	
Y	GB, A, 2 084 608 (SOCIETE NATIONAL ELF AQUITAINE) 15 April 1982 & BE, 390377 FR, 2490672 SE, 8105540 DE, 3137020 NL, 8104298 JP, 57086289 AU, 75288/81 AU, 527434 OA, 6902 US, 4401762 CA, 1156574 JP, 59066882 US, 4460692 CH, 653362 FR, 2512057	1-3
Y	US, A, 4 166 006 (CORNING GLASS WORKS) 28 August 1979	1-3
X	GB, A, 1 604 350 (DOW CORNING CORPORATION) 9 December 1981 & US, 4122029 NL, 7807912 FR, 2398537 DE, 2829388 JP, 54024959 AU, 34875/78 CA, 1093234 AU, 516853	4-6

**This Page is Inserted by IFW Indexing and Scanning  
Operations and is not part of the Official Record**

**BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☒ **BLACK BORDERS**
- ☐ **IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**
- ☐ **FADED TEXT OR DRAWING**
- ☐ **BLURRED OR ILLEGIBLE TEXT OR DRAWING**
- ☐ **SKEWED/SLANTED IMAGES**
- ☐ **COLOR OR BLACK AND WHITE PHOTOGRAPHS**
- ☐ **GRAY SCALE DOCUMENTS**
- ☐ **LINES OR MARKS ON ORIGINAL DOCUMENT**
- ☐ **REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**
- ☐ **OTHER: \_\_\_\_\_**

**IMAGES ARE BEST AVAILABLE COPY.**

**As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.**